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FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE REV. 2/01T **TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371**		ATTORNEY'S DOCKET NUMBER 08364.0022 U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/890855
INTERNATIONAL APPLICATION NO. PCT/GB00/00378	INTERNATIONAL FILING DATE February 8, 2000	PRIORITY DATE CLAIMED February 8, 1999
TITLE OF INVENTION APPARATUS FOR PRESERVING MICROORGANISMS		
APPLICANT(S) FOR DO/EO/US Neil PORTER and Frances Mary GIAQUINTO		

Applicant(s) herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. X The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. X A copy of the International Application as filed (35 U.S.C. 371 (c)(2)).
   - a. X is attached hereto (required only if not communicated by the International Bureau.
   - b. X has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed with the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
   - a. ☐ is attached hereto.
   - b. ☐ has been previously submitted under 35 U.S.C. 154 (d)(4).
7. X Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)).
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. X have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A Substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154 (d)(4).
19. ☐ A second copy of the English language translation of the international application 35 U.S.C. 154 (d)(4).
20. X Other items or information:
   - a. X Copy of cover page of International Publication No. WO 00/47709
   - b. ☐ Copy of Notification of Missing Requirements.
   - c. ☐

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U.S. APPLICATION NO. (If known, see 37CFR 1.5) 09/890855	INTERNATIONAL APPLICATION NO. PCT/GB00/00378	ATTORNEY'S DOCKET NUMBER 08364.0022

21. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTOand all claims satisfied provisions of PCT Article 33 (1)-(4) **$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =					$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).					$	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	43	- 20 =	23	x $18.00	$414.00	
Independent Claims	3	-3 =		x $80.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+$270.00	$270.00	
TOTAL OF THE ABOVE CALCULATIONS =					$1544.00	
X Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.					$772.00	
SUBTOTAL =					$772.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest priority date (37 CFR 1.492(f)).					$	
TOTAL NATIONAL FEE =					772.00	
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +					$	
TOTAL FEES ENCLOSED =					$772.00	
					Amount to be refunded:	$
					charged:	$

a. X A check in the amount of $ 772.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. ________ in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-0916. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
1300 I Street, N.W.
Washington, D.C. 20005-3315

SIGNATURE

Ernest F. Chapman Reg.No. 25,961
NAME/REGISTRATION NO.

DATED: August 7, 2001

181037v1

09 Rec'd PCT/PTO 31 OCT 2001

PATENT
Customer No. 22,852
Attorney Docket No. 08364.0022

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
Neil PORTER et al.	)	Group Art Unit: Unknown
Serial No.: 09/890,855	)	Examiner: Unknown
Filed: August 7, 2001	)	
For: APPARATUS FOR PRESERVING MICROORGANISMS	)	

Being a U.S. National Phase Application based on PCT/GB00/00378

Assistant Commissioner for Patents
Washington, DC 20231

BOX: PCT

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE ABSTRACT:

Please replace the original abstract with the attached abstract of the disclosure numbered as page 45 and attached as the last page of this amendment

IN THE SPECIFICATION:

Page 27, please replace the paragraph beginning on line 6 with the following paragraph:

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Although the embodiment illustrated in Figures 3 to 14 demonstrates how a single receptacle 30 can be connected to another receptacle 30 on a temporary basis to allow for the propagation of a microorganism through growth medium, the invention also contemplates the arrangement illustrated in Figure 15. In that arrangement, a plurality of receptacles 30 of various lengths are connected together by means of collars 54, each receptacle 30 has an insert 40 as previously described and the end receptacles are closed by means of caps 36.

IN THE CLAIMS:

Please amend claims 1, 2, 4, 6-14, 17-26, 28-31, 33-38, 40, and 41 as follows:

1. (Amended) A process for growing an organism, comprising:

providing a first vessel containing a first body of growth supporting material and causing the organism to grow in said material in said first vessel towards a first location;

providing a second vessel containing a second body of growth supporting material; and

permitting said organism to grow from the body of material in the first vessel into the body of material in the second vessel through said first location.

2. (Amended) A process according to claim 1, comprising connecting said second vessel to said first vessel and permitting said organism to grow into said second body of material while said vessels are connected together.

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4. (Amended) A process according to claim 2, comprising disconnecting said first vessel from said second vessel after said organism has begun to grow in said second body of material.

6. (Amended) A process according to claim 1 wherein said step of causing said organism to grow comprises causing said organism to grow in said first vessel and into said second vessel in a predetermined growing direction.

7. (Amended) A process according to claim 6 wherein said first and second vessels are, in the predetermined direction, of different lengths.

8. (Amended) A process according to claim 7 wherein said second vessel is shorter than said first vessel, said process further comprising removing said second vessel, after said step of permitting said organism to grow therein, for sub-sampling thereof.

9. (Amended) A process according to claim 1, comprising causing said organism to grow in said second body of material towards a second location therein, providing a third vessel containing a third body of growth supporting material and permitting said organism to grow from said second body of material into said third body of material through said second location.

10. (Amended) A process of storing a microorganism comprising the steps of:

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providing a growth medium;

growing a population of a microorganism on or in the growth medium; and sampling said population for subculture,

wherein said step of sampling includes sampling across substantially the whole population of the microorganism.

11. (Amended) A process of storing a microorganism including the steps of:

providing a growth medium; and

causing a microorganism to grow on or in the growth medium, wherein said step of causing said organism to grow includes entraining said microorganism substantially in a predetermined direction.

12. (Amended) A process in accordance with claim 11 wherein said step of causing said organism to grow includes causing said organism to grow towards a predetermined location, and said process further comprising sampling at said predetermined location to obtain a sample of said organism across substantially the entire population thereof.

13. (Amended) A process in accordance with claim 12, wherein said sampling step includes the step of placing a sampling medium adjacent said predetermined location for continuing growth of said microorganism thereon or therein.

14. (Amended) A process of manufacturing a metabolite comprising the steps of:

storing a microorganism in accordance with the process of claim 11;

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extracting a sample of said microorganism;

subjecting said sample to conditions suitable for metabolism; and

extracting metabolite from said sample.

17. (Amended) A storage device comprising a housing, growth medium within the housing, and first and second locations on the growth medium, such that a microorganism can be grown from the first location towards the second location where subculturing of substantially the entire population can be effected.

18. (Amended) A storage device in accordance with claim 17, wherein the housing is tubular.

19. (Amended) A storage device in accordance with claim 18, wherein the housing is cylindrical.

20. (Amended) A storage device in accordance with claim 17 wherein the housing is of a sterilizable material.

21. A storage device in accordance with claim 17 wherein said housing has formations at said first and second locations of the growth medium each formation being suitable to engage with a cooperating formation of another of said storage device, for propagation of microorganism therebetween.

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22. (Amended) A storage device in accordance with claim 17 wherein said housing comprises means for retaining said growth medium.

23. (Amended) A storage device in accordance with claim 22 wherein said retaining means has at least one aperture defined therein for passage of microorganism therethrough.

24. (Amended) A storage device in accordance with claim 23 wherein said retaining means comprises at least one retaining member across said retaining means.

25. (Amended) A storage device in accordance with claim 24 wherein said retaining means comprises a reticular member across said retaining means.

26. (Amended) A storage device in accordance with claim 17 wherein said growth medium comprises a natural foodstuff.

28. (Amended) A storage device in accordance with claim 27 wherein said growth medium comprises a quantity of a cereal.

29. (Amended) A storage device in accordance with claim 27 wherein said growth medium comprises a quantity of seed.

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30. (Amended) A storage device in accordance with claim 27 wherein said growth medium comprises a quantity of a pulse.

31. (Amended) A storage device in accordance with claim 27 wherein said growth medium comprises an agricultural crop byproduct.

33. (Amended) A storage device in accordance with claim 26 wherein said growth medium comprises at least one of calcium sulphate, soy oil, yeast extract and peptone.

34. (Amended) A storage device in accordance with claim 17 wherein said growth medium is sterile.

35. (Amended) A storage container for use in the storage device of claim 17, defining a cavity within which growth medium can be contained, said container comprising first and second access means between which growth medium can extend in use, for growth of an organism between said first and second access means in use.

36. (Amended) A container in accordance with claim 35 including first and second closure means, removably closing said access means in use.

37. (Amended) A storage device comprising:

growth medium for viably supporting a microorganism;

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wherein said storage device includes a facility for presenting said population substantially in its entirety for subculture.

38. (Amended) A storage device in accordance with claim 37 and further comprising a receptacle supporting the growth medium.

40. (Amended) A storage device in accordance with claim 38, wherein the receptacle includes attachment means for attachment of said device to further culturing apparatus.

41. (Amended) A storage device in accordance with claim 40, wherein the attachment means is operable to engage the growth medium with growth medium of a further storage device.

REMARKS

The specification has been amended at page 27 to conform the specification to the drawings. The foregoing amendments to the claims have been made to conform them to customary U.S. practice.

Attached hereto is a marked-up version of the changes made to the specification and claims by this Preliminary Amendment. The attachment is captioned "**APPENDIX TO PRELIMINARY AMENDMENT OF OCTOBER 31, 2001**" Deletions appear as normal text surrounded by [ ] and additions appear as underlined text.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

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Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: October 31, 2001

By: for Jeffrey A. Berkowitz
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APPENDIX TO PRELIMINARY AMENDMENT OF OCTOBER 31, 2001

Amendments to the Abstract:

[ABSTRACT]

ABSTRACT OF THE DISCLOSURE

APPARATUS FOR PRESERVING MICROORGANISMS

Subculturing apparatus [(30)] comprises a receptacle for supporting a growth medium [(5)] for culturing of a microorganism. The microorganism is entrained to grow in a predetermined direction, towards an end of the growth medium [(50)], where a further apparatus [(30)] can be placed adjacent thereto. The microorganism can then grow into the growth medium of the further apparatus. The method of storing a microorganism for use in microbiological processes is also described, as is a method of fermentation of a stored microorganism for the production of biochemicals such as pharmaceuticals or agrochemicals.

[(Figure 4)]

Amendments to the Specification:

Page 27, the paragraph beginning on line 6:

Although the embodiment illustrated in Figures 3 to 14 demonstrates how a single receptacle 30 can be connected to another receptacle 30 on a temporary basis to allow for the propagation of a microorganism through growth medium, the invention also

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contemplates the arrangement illustrated in Figure 15. In that arrangement, a plurality of receptacles 30 of various lengths are connected together by means of collars [56] 54, each receptacle 30 has an insert 40 as previously described and the end receptacles are closed by means of caps 36.

Amendments to the Claims:

1. (Amended) A process for growing an organism, comprising:

providing a first vessel [(30)] containing a first body of growth supporting material [(50)] and causing the organism to grow in said material in said first vessel towards a first location;

providing a second vessel [(30)] containing a second body of growth supporting material [(50)]; and

permitting said organism to grow from the body of material [(50)] in the first vessel [(30)] into the body of material [(50)] in the second vessel [(30)] through said first location.

2. (Amended) A process according to claim 1, comprising connecting said second vessel [(30)] to said first vessel [(30)] and permitting said organism to grow into said second body [(50)] of material while said vessels are connected together.

4. (Amended) A process according to claim 2 [or claim 3], comprising disconnecting said first vessel [(30)] from said second vessel [(30)] after said organism has begun to grow in said second body of material.

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6. (Amended) A process according to [any one of] claim[s] 1 [to 5] wherein said step of causing said organism to grow comprises causing said organism to grow in said first vessel [(30)] and into said second vessel [(30)] in a predetermined growing direction.

7. (Amended) A process according to claim 6 wherein said first and second vessels [(30)] are, in the predetermined direction, of different lengths.

8. (Amended) A process according to claim 7 wherein said second vessel [(30)] is shorter than said first vessel [(30)], said process further comprising [said step of] removing said second vessel, after said step of permitting said organism to grow therein, for sub-sampling thereof.

9. (Amended) A process according to [any one of] claim[s] 1 [to 8], comprising causing said organism to grow in said second body of material [(50)] towards a second location therein, providing a third vessel [(30)] containing a third body of growth supporting material [(50)] and permitting said organism to grow from said second body of material [(50)] into said third body of material [(50)] through said second location.

10. (Amended) A process of storing a microorganism comprising the steps of:

providing a growth medium [(50)];

growing a population of a microorganism on or in the growth medium [(50)]; and

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sampling said population for subculture[;],

[characterised in that] wherein said step of sampling includes sampling across substantially the whole population of the microorganism.

11. (Amended) A process of storing a microorganism including the steps of:

providing a growth medium [(50)]; and

causing a microorganism to grow on or in the growth medium[;], [characterised in that] wherein said step of causing said organism to grow includes [training] entraining said microorganism substantially in a predetermined direction.

12. (Amended) A process in accordance with claim 11 wherein said step of causing said organism to grow includes causing said organism to grow towards a predetermined location, and said [method] process further comprising sampling at said predetermined location to obtain a sample of said organism across substantially the entire population thereof.

13. (Amended) A process in accordance with claim 12, wherein said sampling step includes the step of placing a sampling medium [(50)] adjacent said predetermined location for continuing growth of said microorganism thereon or therein.

14. (Amended) A process of manufacturing a metabolite comprising the steps of:

storing a microorganism in accordance with the [method] process of [any of claims 1 to 13] claim 11;

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extracting a sample of said microorganism;

subjecting said sample to conditions suitable for metabolism; and

extracting metabolite from said sample.

17. (Amended) A storage device [for use in the process of any of claims 1 to 15 including] <u>comprising</u> a housing [(30)], growth medium [(50)] within the housing [(32)], and first and second locations on the growth medium, such that a microorganism can be grown from the first location towards the second location where subculturing of substantially the entire population can be effected.

18. (Amended) A storage device in accordance with claim 17, wherein the housing [(32)] is tubular.

19. (Amended) A storage device in accordance with claim 18, wherein the housing [(32)] is cylindrical.

20. (Amended) A storage device in accordance with [any one of] claim[s] 17 [to 19] wherein the housing [(32)] is of a sterilizable material.

21. A storage device in accordance with [any one of] claim[s] 17 [to 20] wherein said housing [(32)] has formations [(34)] at said first and second locations of the growth medium each formation being suitable to engage with a cooperating formation of another of said storage device [(30)], for propagation of microorganism therebetween.

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22. (Amended) A storage device in accordance with [any one of] claim[s] 17 [to 21] wherein said housing [(32)] comprises means [(40)] for retaining said growth medium.

23. (Amended) A storage device in accordance with claim 22 wherein said retaining means [(40)] has at least one aperture defined therein for passage of microorganism therethrough.

24. (Amended) A storage device in accordance with claim 23 wherein said retaining means [(40)] comprises at least one retaining member [(42')] across said [vessel] retaining means.

25. (Amended) A storage device in accordance with claim 24 wherein said retaining means [(40)] comprises a reticular member [(42)] across said [vessel] retaining means.

26. (Amended) A storage device in accordance with [any one of] claim[s] 17 [to 25] wherein said growth medium [(50)] comprises a natural foodstuff.

28. (Amended) A storage device in accordance with claim 27 wherein said growth medium [(50)] comprises a quantity of a cereal.

29. (Amended) A storage device in accordance with claim 27 [or claim 28] wherein said growth medium [(50)] comprises a quantity of seed.

30. (Amended) A storage device in accordance with [any one of] claim[s] 27[, 28 or 29] wherein said growth medium [(50)] comprises a quantity of a pulse.

31. (Amended) A storage device in accordance with [any one of] claim[s] 27 [to 30] wherein said growth medium [(50)] comprises an agricultural crop byproduct.

33. (Amended) A storage device in accordance with [any one of] claim[s] 26 [to 32] wherein said growth medium [(50)] comprises at least one of calcium sulphate, soy oil, yeast extract and peptone.

34. (Amended) A storage device in accordance with [any one of] claim[s] 17 [to 33] wherein said growth medium [(50)] is sterile.

35. (Amended) A storage container for use in the storage device [(30)] of [any one of] claim[s] 17 [to 35], defining a cavity within which growth medium can be contained, said container [(32)] comprising first and second access means between which growth medium can extend in use, for growth of an organism between said first and second access means in use.

36. (Amended) A container in accordance with claim 35 including first and second closure means [(30)], removably closing said access means in use.

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37. (Amended) A storage device [for use in the process of any one of claims 1 to 15] comprising:

growth medium [(50)] for viably supporting a microorganism;

[characterised in that]

wherein said storage device includes a facility for presenting said population substantially in its entirety for subculture.

38. (Amended) A storage device in accordance with claim 37 and further comprising a receptacle [(32)] supporting the growth medium.

40. (Amended) A storage device in accordance with claim 38 [or claim 39], wherein the receptacle [(32)] includes attachment means [(34)] for attachment of said device to further culturing apparatus.

41. (Amended) A storage device in accordance with claim 40, wherein the attachment means [(34)] is operable to engage the growth medium with growth medium of a further storage device [in accordance with any one of claims 37 to 40

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ABSTRACT OF THE DISCLOSURE

APPARATUS FOR PRESERVING MICROORGANISMS

Subculturing apparatus comprises a receptacle for supporting a growth medium for culturing of a microorganism. The microorganism is entrained to grow in a predetermined direction, towards an end of the growth medium, where a further apparatus can be placed adjacent thereto. The microorganism can then grow into the growth medium of the further apparatus. The method of storing a microorganism for use in microbiological processes is also described, as is a method of fermentation of a stored microorganism for the production of biochemicals such as pharmaceuticals or agrochemicals.

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APPARATUS FOR PRESERVING MICROORGANISMS

The present invention relates to preparing and maintaining cultures of microorganisms, and is particularly applicable to fungi and bacteria.

Microorganisms are essential to many important biotechnical processes including the production of foods, fine chemicals such as vitamins and organic acids, pharmaceuticals, enzymes, agrochemicals and biological control agents. In the pharmaceutical field, microorganisms have yielded drugs which are used for treating infections, disorders of the central nervous system, cardiovascular disease and for suppression of the immune system to prevent rejection following organ transplantation. They hold enormous potential for producing new pharmaceutical compounds.

As a consequence of the economic and academic importance of microorganisms, microbial genetic resource collections have been established to provide cultures of microorganisms for research. The World Data Center for Microorganisms has been assigned the role of characterising, cataloguing and most importantly preserving microorganisms in a pure, viable and

genetically stable condition. Over 500 collections of microorganisms have been registered with the World Data Center.

In industry, where collections can exceed 50,000 microorganisms, and each has the potential to yield a valuable new product or process, it is imperative that microorganism cultures are stored under conditions that maintain genetic stability. Genetic deterioration of a microorganism during storage can result in a reduction or total loss of its biotechnological properties (known or as yet undiscovered). This can result in a significant financial loss to a company.

There are two main approaches to microbial preservation. Firstly, a culture can be maintained on a growth substrate by means of repeated sub-culture onto a new substrate as the growth substrate deteriorates. Secondly, it is possible to create an environment where metabolism of a culture is severely reduced or halted (Smith, D & Onions, AHS, (1994) "The Preservation and Maintenance of Living Fungi", 2nd ed. Wallingford, CAB International).

A method of sub-culturing will now be described with

reference to Figures 1 and 2 of the drawings.

Figure 1 shows a Petri dish 10 which is filled with a layer of agar 12 through which is dispersed a nutrient medium containing sources of carbon, nitrogen, phosphorus, essential vitamins and other elements required for growth. Agar is a natural carbohydrate substance extracted from seaweed. A sample of an organism 14 is inoculated onto the agar at the centre of the Petri dish 10. The Petri dish 10 is then left in a clean environment for a period of 10 days to 2 weeks, or longer if necessary, and maintained at a temperature suitable to promote growth, e.g. 15-25°C. Following that period, the dish will have the appearance as illustrated in Figure 2. As illustrated in Figure 2, a colony of the organism 14 has developed by the growth of filamentous strands along the surface of the agar 12 in all directions from the original sample.

Then, as illustrated in Figure 2, a sub-culture sample 16 can be taken from the growing edge, so as to sample the youngest and most viable part of the filaments. That sub-culture sample can then be inoculated at the centre of a further Petri dish of agar for further culturing. The sample 16 can be taken by means of a sterile scalpel.

The original Petri dish can then be discarded.

It has been recognised that the above method can lead to sampling errors. Microorganisms, and particularly fungi, are inherently genetically variable. For example, as illustrated in Figure 2, whereas the microorganism normally has a green appearance, the zone 18 of the culture identified by chain lines could have genetically segregated so that it has a red appearance. This phenomenon is known as sectoring.

By taking a sample 16 from the Petri dish as illustrated, only genetically segregated red material would be taken from the Petri dish. Therefore, the process of sub-culturing as described above would, under those circumstances, result in the sub-cultured sample having a different overall genetic make-up from the culture from which the sub-culture was selected. It could be that only the green part of the culture exhibited the biological and/or physiological features which might have an advantage suitable for pharmaceutical or agrochemical application. Therefore, by only taking the one sample, the benefit might have been eliminated. This problem was identified in Smith & Onions (1994), referred to above.

Accordingly, a further sample 16' could be taken as illustrated in Figure 2. This second sample 16' would maintain the full genetic composition of the microorganism, which would reduce the problems resulting from genetic segregation. However, some sampling error may remain, since a technologist may not be able to identify all genetic modifications by observation, and so important genetic material may be discarded as a result of the sub-culturing method described above. Moreover, the proportion of materials of different types to be sampled would be a matter for a sampler to identify, which could introduce further errors.

Additionally, it should be emphasised that all microorganism populations are genetically heterogeneous. Consequently, ongoing sub-culture and growth on a synthetic agar medium can act as a selective pressure ensuring that a proportion of the population best suited to those particular conditions of growth become dominant. Desirable properties of microorganisms can be lost as a result. Long term storage of microorganisms by repeated sub-culture is therefore not desirable.

Avoiding repeated sub-culture by covering cultures grown on agar slopes with mineral oil is a traditional method

still widely used. The mineral oil (liquid paraffin) prevents dehydration and slows down metabolism by reducing oxygen availability (Smith & Onions 1994 previously identified; Smith, D & Kolkowski, J (1996) "Preservation and Maintenance of Cultures used in Biotechnology and Industry", San Diego, CA Academic Press). Although fungi have been successfully stored for 40 years using this method, it has a number of serious disadvantages which include retarded growth of the microorganism on retrieval and an increased risk of contamination.

One of the most widely used methods of creating an environment for storing microorganisms in such a way that their metabolic rate is reduced or halted involves the use of cryopreservation at ultra-low temperatures (Smith, D (1993) "Tolerance to freezing and thawing", Tolerance of Fungi, Editor - Jennings, DH pp 145-171 published by Marcel Dekker Inc, New York Smith, D (1998) "The use of cryopreservation in the ex-situ conservation of fungi" - Cryoletters 19, 79-90). Little metabolic activity occurs below -70°C but recrystallisation of ice, which can cause cell damage, can occur above -130°C. Consequently, microorganisms are stored at temperatures below -130°C; in refrigerators (-135°C to -180°C) or in liquid

nitrogen vapour at -196°C. Cellular damage due to ice crystal formation can occur if the freezing and thawing rates are not carefully controlled and the use of cryoprotectant chemicals is important to minimise this damage (Smith (1998) previously referred to; Smith D & Thomas VE (1998) "Cryogenic light microscopy and the development of cooling protocols for the cryopreservation of filamentous fungi", World Journal of Microbiology and Biotechnology, 14, 49-57).

Genetic selection can also be a problem with this method of storage; only a small amount of culture biomass is taken for cryopreservation and only a proportion of that small amount may be viable when the material is thawed.

Cryopreservation is the most expensive in terms of capital equipment required, its running costs and preparation of cultures for storage.

For organisms that sporulate in culture, various methods of drying and freeze drying can be employed. Removal of water reduces cell metabolism and many fungal spores can remain dormant but viable in this way for a number of years. Storage in silica gel is a cheap and effective method for fungi that produce thick walled spores and it

maintains good genetic stability. Storage in sterilised soil is used successfully for some soil fungi but loss of genetic integrity is common and there is a high risk of contamination. Freeze drying involves removing water from frozen cell suspensions by sublimation under reduced pressure (Mellor J.D. "Fundamentals of Freeze Drying", Academic Press 1978). It is a widely used method, but it is unsuitable for non-sporulating fungi, there is often a low percentage viability, genetic damage frequently occurs and it requires expensive equipment.

The time taken for microorganisms to recover from storage where their cellular metabolic activity has been reduced can take three weeks or longer. It is not possible to do anything with the microorganism before the end of this period. In addition to the inconvenience caused, this time delay can add significantly to the costs of biotechnological processes.

There is a need for a simple, cost effective system for the storage and sub-culture of microorganisms which maintains cultures in a viable, metabolically active and genetically stable state.

Therefore, a first aspect of the invention has as its

object the improvement of sampling techniques to maintain reliability of sub-culturing as a method of maintaining a sample of an organism.

Moreover, the use of agar is somewhat undesirable, in that it is a synthetic growth environment which is in some respects different from the nutrient environment which microorganisms would naturally encounter. Whereas agar media are designed to simulate, as closely as possible, the combination of nutrients most amenable to the growth of microorganisms, they remain approximations. The main advantage of agar is that it provides a solid substrate that is not broken down by the microorganism.

Therefore, it is a further object of an aspect of the invention to provide a technique of culturing which makes use of naturally occurring substrates.

The invention provides, in a first aspect, a method of sub-culturing which involves maintenance of microbiological material without selection of specific samples thereof.

The invention provides, in a second aspect, sub-culturing apparatus for presenting a sub-culture across

substantially an entire population of a culture.

The invention provides, in a third aspect, a method of generating a metabolite from an organism maintained according to the first aspect of the invention. The invention also provides, in a further aspect, a method of manufacturing a chemical composition from the metabolite generated in accordance with the third aspect of the invention.

A specific embodiment of the invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a perspective view of a Petri dish in accordance with an example of an existing technique;

Figure 2 is a plan view of a culture on the Petri dish illustrated in Figure 1;

Figure 3 is a perspective view of a receptacle in accordance with a specific embodiment of the present invention;

Figure 4 is a longitudinal section of the receptacle

illustrated in Figure 3 in an initial condition;

Figure 5 is a perspective view of an insert of the receptacle illustrated in Figure 3;

Figure 6 is a perspective view of an alternative insert to that illustrated in Figure 5;

Figure 7 shows a longitudinal section of an end portion of receptacle in accordance with an alternative and specific embodiment of the invention;

Figure 8 is a perspective view of a receptacle of a further alternative and specific embodiment of the present invention;

Figure 9 is a longitudinal sectional view of the receptacle illustrated in Figure 3 in a first stage of use in accordance with a specific exemplary method;

Figure 10 is a longitudinal sectional view of the receptacle illustrated in Figure 3 in a second stage of use in accordance with a specific exemplary method;

Figure 11 is a longitudinal sectional view of the

receptacle illustrated in Figure 3 in a third stage of use in accordance with a specific exemplary method;

Figure 12 is a longitudinal sectional view of the receptacle illustrated in Figure 3 in a fourth stage of use in accordance with a specific exemplary method;

Figure 13 is a longitudinal sectional view of the receptacle illustrated in Figure 3 in a fifth stage of use in accordance with a specific exemplary method;

Figure 14 is a longitudinal sectional view of the receptacle illustrated in Figure 3 in a sixth stage of use in accordance with a specific exemplary method; and

Figure 15 is a longitudinal sectional view of an arrangement of receptacles as illustrated in Figure 3 for use in accordance with an alternative specific exemplary method.

Referring to Figure 3, a receptacle 30 has a generally hollow cylindrical body 32 which is open at both ends. As shown in Figure 4, each end of the body 32 has an external screw thread 34, and is closed by a cap 36 having a cooperating internal screw thread 38. The caps

36 may be fitted on the body 32 sufficiently tightly that a seal is formed to prevent ingress of microscopic contaminants into the receptacle 30.

The material of the body 32 and the caps 36 is of material which is readily sterilisable for use in biotechnological applications. Moreover, the material is preferably transparent, which allows for observation of the interior of the receptacle 30. A suitable material could be glass, or plastics such as polystyrene, polyethylene, polyamide, polyacrylate. Especially important examples of a suitable material are polycarbonate or polypropylene, which can withstand sterilisation by means of hot water vapour at temperatures up to 121°C.

Each cap 36 extends over its respective end of the body 32 to a depth of not less than 25 mm to ensure that the ends of the body remain sterile when one or both of the caps 36 are removed.

The thickness of the wall of the cylindrical body 32 is 1 mm. Alternative embodiments may have thicker walls, for instance 4 mm, but it is preferable that the wall is sufficiently transparent that the contents of the

receptacle 30 can be observed therethrough. Moreover, the body may be of any other suitable shape other than that of a cylinder.

The internal diameter of the body 32 is 22mm, although this can be varied in alternative embodiments, for example up to 100 mm. Moreover, in the present example, the body 32 is 90 mm in length, but other lengths of body 32 are also envisaged, for instance 50, 150 or 250 mm.

An insert 40, as illustrated in Figure 5, comprises a fine mesh 42, supported on a circular collar 44. The insert 40 is placed at one end of the interior of the body 32 (the right hand end as illustrated in Figure 4). The mesh 42 retains the contents of the receptacle 30 in the event of removal of the cap 36 at that end. The collar 44 is of a size suitable for it to form a tight fit within the body 32, to reduce the risk of the insert 40 accidentally falling out of place. The mesh 42 is sufficiently fine as to prevent egress of any growth medium contained in the receptacle therethrough, but not so fine that a filamentous microorganism is impeded from growing therethrough.

Figure 6 shows an alternative insert 40' having two crosspieces 42' supported on a collar 44'. The collar 44' is identical with the collar 44 illustrated in Figure 5. The crosspieces 42' extend diametrically and mutually perpendicularly across the collar 44'. The crosspieces 42' are operative, in use, to retain any contents of the receptacle 30 to the extent that the cap 36 at that end can be removed and replaced without significant shifting of the medium between the crosspieces 42'. It will be understood that the crosspieces 42' act to impede bulk movement rather than actively preventing it.

The use of an insert 40' with crosspieces 42' as illustrated in Figure 6 is particularly appropriate where the receptacle 30 is used to contain a highly particulate growth medium.

An alternative example of a receptacle 30' including a push-type fitting between a body 32' and a cap 36' is illustrated in Figure 7 of the drawings. In that example, the second embodiment of the insert 40', as illustrated in Figure 6, has been fitted at the end of the body 32'. The end of the body 32' is tapered on its exterior surface, and a corresponding interior tapered surface is formed on the cap 36'. The cap 36' can then

be urged onto the end of the body 32' and, by means of friction and selection of suitable taper angles, the cap 36' can be retained on the body 32', forming a tight seal. Other than this push-fit lid fitting arrangement, alternative arrangements are also envisaged, for example a bayonet fitting, and a push and twist fitting.

An alternative embodiment of the receptacle 30' illustrated in part in Figure 7 is further illustrated in Figure 8. A rectangular membrane 46' is incorporated into the wall of the body 32'. The membrane 46' is of a hydrophobic material, such as polytetrafluoroethylene or polysiloxane, which allows the transfer of gases therethrough, for example oxygen, which is required in many circumstances for the growth of microorganisms. Alternatively, or in addition, the membrane may be located within one or other of the caps 36'. As noted with respect to other components of the receptacle 30', the material selected for the membrane 46' should be suitable for withstanding sterilisation by means of hot water vapour at temperatures up to 121°C.

The membrane extends along the length of the body 32', up to a distance of 25 mm from each end of the body 32'.

With reference to Figures 9 to 14, a specific method of storing a microorganism will now be described.

As illustrated in Figure 9, the receptacle 30 previously described is filled with a suitable growing medium 50. A growing medium should contain assimilable sources of carbon, nitrogen and mineral salts.

Assimilable sources of carbon, nitrogen and minerals may be provided by simple or complex nutrient sources. Preferably, complex nutrient sources are used since they reflect more accurately the natural substrates on which the microorganisms grow. The great variety of nutrients present in complex sources may prevent the unwanted selection of genetic variants existing in a microorganism population which can occur by the placement of the population in an unnatural environment.

Complex sources of carbon, nitrogen and minerals may be provided by clean (not containing chemical residues such as fungicides or other pesticides) grains, cereals and seeds. Examples of such sources are tabulated below:

Cereals and grains	Seeds	Pulses	Agricultural waste	Other
Quinoa	Rye grass	Aduki beans	Ground corn cobs	Wood chips
Maize	Sunflower	Whole lentils	Peanut shells	Wheat bran
Millet	Linseed	Soya	Tea leaves	Oat bran
Rye			Straw (especially from wheat, rye, rice, oat and sorghum)	Other brans including corn, soybean, rye, rice
Wheat				Sawdust (especially hardwood)
Oats				Soil
Rice				Peat moss

Ideally, a medium is formed of a mixture of the above materials, to provide optimum conditions for microorganism storage. Moreover, supplements may be added to the mixture, for example calcium sulphate (which separates individual grains), soy oil, yeast extract or peptone. Peptone is a hydrolysed protein which can originate from animal or plant products.

For example, for basidiomycetes such as *Schizospora paradoxa*, collected and isolated from British woodland, a medium consisting of quinoa is suggested. The use of

the receptacle 30 to store a sample of that microorganism will now be described with reference to Figures 9 to 14 of the drawings.

Quinoa is soaked in boiling water, in the proportion of 1 kg quinoa to 1 litre of water. The mixture is left until it has absorbed all of the water. Then, the receptacle 30 is filled with the soaked mixture to a density of 0.8 g/cm^3. Generally, a density within the range 0.6 - 1.0 g/cm^3 would be acceptable.

Excessive compression of the mixture could inhibit filamentous growth of the microorganism, which could lead to differentiation of the microorganism. Insufficient compression could lead to voids appearing in the growth medium as water and medium is consumed by the microorganism, which would result in unsatisfactory growth of the microorganism. Once the receptacle 30 has been filled with the quinoa medium to the appropriate density and sealed by fitting the cap 36, the whole unit is sterilised by exposure to hot water vapour at 121°C for 40 minutes.

A new microorganism population is inoculated into the left hand end of the growth medium 50 as illustrated in Figure 9, i.e. the end not made inaccessible by the insert 40. Inoculation is effected by aseptically placing a sample 52 taken from an originating population grown in agar, or on grain or other nutrient source, and directly placing that sample 52 into the growth medium 50.

Inoculation could also be effected by injection of a liquid carrying the microorganism into the growth medium 50.

Once inoculation has taken place, the receptacle 30 is placed in conditions that allow optimum growth of the microorganism. These conditions include temperatures ranging from 10°C to 27°C, but ideally 18-25°C for filamentous fungi, in a clean environment such as a specially designed growth cabinet room or incubator so as to minimise risk of contamination from other microorganisms or invertebrate pests such as mites. The incubation conditions can include humidity and light

regulation. A moderately humid environment will reduce the risk of medium in each receptacle from drying out. Sporulation of some microorganisms is induced by diurnal light cycles so light regulation may be advantageous if sporulation of a stored microorganism is required. Figure 10 illustrates the expected appearance of a receptacle 30 after this step has been performed.

Once colonisation of the receptacle 30 by the microorganism has been initiated the receptacle is transferred to storage conditions that reduce growth to a minimum. These conditions can be created in purposefully designed storage cabinets, rooms or incubators to provide clean conditions so as to minimise risk of contamination from other microorganisms or invertebrate pests such as mites. The temperature required to reduce growth should be above freezing and may vary from 4°C to 12°C but ideally 6-10°C. The storage conditions may include humidity and light regulation. Figure 11 shows a receptacle 30 after storage thereof for a period of about six months.

When, as shown in Figure 11, the filamentous growth of the microorganism is nearing the end of the receptacle 30 in which is placed the insert 40, the cap 36 at that end is removed, and a left hand end of a further receptacle 30 is placed in abutment with that end. As shown in Figure 12, in order to retain the receptacles 30 in abutment, a collar 54 having internal thread 56 cooperable with the external threads 34 of the bodies 32 is engaged with those external threads 34. At this stage it should be noted that there may be benefit in making the threads at each end of the bodies 32 of opposite sense, i.e. one being left handed and the other being right handed. In that way, a simple twisting action of the collar 54 is sufficient in one direction to draw the receptacles 30 into close abutment, or in the opposite direction to urge the receptacles 30 apart.

Close abutment of the receptacles 30 is preferred, particularly because the growth medium 50 of the further receptacle 30 should contact the insert 40 of the original receptacle 30. In that way, the filamentous growth of the microorganism may continue uninterrupted.

Once filamentous growth has established itself in the further receptacle 30 as shown in Figure 13, the original receptacle is discarded and a sterile cap 36 is placed at the left hand end of the further receptacle. The further receptacle 30 is then stored in the same way, which will result in a filamentous microorganism as illustrated in Figure 14.

The present invention is particularly applicable to organisms which do not produce a resting state such as spores. For these organisms, such as the basidiomycotina, ascomycotina and other sterile mycelia, the mycelium is the source of inoculum. Therefore, by collecting the youngest part of the colony, as a whole, in a further receptacle, the most vigorous part of the colony, and therefore the most viable part, is maintained.

A further example will now be described of use of the apparatus illustrated in Figures 3 to 14 for the maintenance and sub-culture of a microorganism. *Phlebia deflectens* is a basidiomycete which is commonly found

growing on rotten deciduous wood in British woodland.

In preparation, the sub-culturing apparatus 30 is filled with a growth medium 50 consisting of swollen quinoa grain to a density of 0.8 gm/cm^3. The quinoa grain is swollen by soaking for twelve hours in boiling deionised water in the proportion 1 kg of grain to 1 litre of water. Once the apparatus is filled, it is sterilised by the application of hot water vapour at 121°C for 40 minutes, followed by cooling under sterile conditions.

The spores of *Phlebia deflectens* are collected from fruit bodies in the autumn and germinated on agar containing suitable nutrients. Once a viable colony has been established on the agar, a sample can be taken from the growing edge of the colony using a sterile scalpel. The sample 52 is placed at one end of the sub-culturing receptacle 30 prepared as described above using standard microbiological techniques under sterile conditions. The apparatus 30 is then labelled and stored at 22°C for four weeks. After this time, it has been observed that mycelium grows uniformly through the medium 50 to a total

length of 15 mm and the apparatus 30 can then be transferred to a controlled dark environment at 10°C. After a further four weeks, it has been observed that the mycelium grows a further 5 to 8 mm. This indicates that a drop of temperature from 22°C to 10°C reduces the growth of the mycelium by at least 50%. After 24 weeks in the controlled dark environment, the mycelium is likely to have reached the end of the growth medium 50 and a further receptacle 30 can then be added for further sub-culture.

Phlebia deflectens is a basidiomycete that does not produce any resting stage, such as spores, in laboratory culture. Moreover, its growth and viability are observed to become curtailed when grown and sub-cultured on agar for several months. The method described above using the sub-culturing receptacle 30 maintains the vigour and viability of the *Phlebia deflectens* microorganism, and rapid growth has been observed when a sub-culture of the mycelium is then transferred to a Petri dish containing agar medium for viability testing.

Phlebia deflectens grows at a moderate rate in the sub-culturing receptacle 30. On the other hand, other basidiomycetes and ascomycetes grow at a faster rate than the above example, for example 250 mm in four weeks at 22°C. For such robust and fast growing microorganisms, a cooler storage temperature of 6°C is necessary to reduce growth sufficiently to prevent the microorganism from reaching the end of the growth medium 50, and thus requiring further sub-culture, at intervals of less than six months. Otherwise, the storage method involving the use of the receptacle 30 would become quite labour intensive.

Moreover, since the apparatus may be of relatively simple construction, it provides an ideal solution to the problem of preservation of a local biology, especially in developing countries. In recent times, ecological campaigns here have resulted in countries becoming more aware of their diverse local biology, and efforts have been made for the preservation thereof. The preservation of local microbiology is an integral part of that process.

The illustrated embodiment can be stored easily on racks within an incubator. No sampling takes place and so there is less danger of a reduction in heterogeneity of a microorganism stored in accordance with the invention.

Although the embodiment illustrated in Figures 3 to 14 demonstrates how a single receptacle 30 can be connected to another receptacle 30 on a temporary basis to allow for the propagation of a microorganism through growth medium, the invention also contemplates the arrangement illustrated in Figure 15. In that arrangement, a plurality of receptacles 30 of various lengths are connected together by means of collars 56, each receptacle 30 has an insert 40 as previously described, and the end receptacles are closed by means of caps 36.

A microorganism as shown in Figure 15 can be allowed to grow from one end of the arrangement towards the other, and samples may be taken from the colony of the microorganism by disconnecting the arrangement and removing one or more of the receptacles 30 as required.

Connection and disconnection of receptacles 30 is to be carried out in a sterile manner, for instance in the presence of a sterile airflow, or in the near vicinity of a naked flame, for example a Bunsen burner flame.

The above described apparatus and procedures for sub-culturing and maintaining microorganisms in a genetically stable state can be used to improve the process of culturing microorganisms in a liquid fermentation system either as a surface culture or as a submerged culture or any other type of fermentation for use in a biotechnological process.

Fermentation procedures normally employ one or more growth or seed stages to increase microbial biomass to a level which can be used to inoculate the final production medium designed to yield optimal levels of a desired metabolite. Inoculation levels of biomass to subsequent fermentations is critical to the optimal growth of a microorganism and overall productivity (grams product/unit biomass/unit time) of the process.

A growth stage is typically initiated by the introduction of a small amount of microorganism, which has been cultured on an agar medium, to a 250 ml Erhlenmeyer flask containing 30-50 ml of liquid growth medium. The organism is then cultivated by agitation at a desired temperature (20-40°C) for a period dependent on the growth rate of the organism (range 2-10 days). This culture volume can be increased by a factor of ten at each stage by transferring to ten times the volume of fresh medium and so on.

The seed stage is used to inoculate production medium in which the organism is cultivated to produce the desired product which may be extracted and purified and which may have pharmaceutical, agrochemical or other properties.

A problem encountered with the above described fermentation process is that several seed stages are required to generate sufficient biomass to inoculate the production medium.

The sub-culturing receptacle 30 described above is

advantageous in that it can be used to generate sufficient microbial biomass for direct inoculation of production medium. The inoculum may be prepared in a number of ways prior to inoculating the production medium, for example:

i) The microbial biomass mixed with substrate (from the sub-culturing apparatus) can be used as a direct inoculum.

ii) The microbial biomass mixed with substrate can be gently agitated with an aliquot of production medium or other suitable liquid medium. This suspension is then allowed to settle or is centrifuged at very low speed so that the heavier solid substrate materials are sedimented and removed leaving a suspension of biomass which is used as an inoculum.

iii) The microbial biomass mixed with substrate can be suspended in an aliquot of production medium or other suitable liquid medium and gently blended

under aseptic conditions using a Waring (or similar) blender. This procedure produces a substantially homogeneous suspension of biomass and substrate with a much higher inoculum potential i.e. the gentle blending step releases all the biomass from the substrate and breaks it up to produce more growing points.

In each case the optimal inoculation level will be in the range 1% to 10% weight of biomass combined with substrate (from sub-culturing apparatus) to volume or weight of reproduction medium. An ideal inoculation level is 3%-5%.

The following example describes the use of microbial biomass generated in the sub-culturing apparatus as a direct inoculum for the production of the pharmaceutical compound mevinolin by the organism *Aspergillus terreus* Thom ATCC 20542.

A terreus can be maintained and sub-cultured using the above described receptacle 30 using the grain quinoa as

a growth medium. As described previously, the receptacle 30 is prepared by mixing quinoa with boiling deionised water and leaving the mixture for 12 hours. The swollen grain is then packed into the sub-culturing receptacle 30 to a density of 0.8 g per cm^3. The organism can be maintained in this growth medium 50 at a temperature of 10°C until required for initiating a fermentation process. By raising the temperature to 25°C, the growth rate of the organism can be increased and multiple sub-cultures made onto fresh grain (in separate sub-culturing receptacles) as a means of quickly generating microbial biomass for inoculation purposes.

For fermentation, a liquid medium A is prepared in accordance with the following composition:

	grams/litre
Sheftone N-Z soy peptone (Sheffield Products)	10
Malt extract (Oxoid L39)	21
Glycerol (Sigma-Aldrich)	40
Deionised water	add and make up to 1 litre
Adjust pH to 6.3 using 2N NaOH/2N HCl	

60 ml of the above medium is transferred to a 250 ml Erhlenmeyer flask, stoppered with a polystyrene foam bung and autoclaved at 121°C for 20 minutes. 20g of *A terreus* culture growing on quinoa substrate is then removed from the sub-culturing receptacle 30, mixed with 20 ml of sterile medium A and aseptically blended (up to five 2 second bursts) using a Waring blender. 6 ml of the homogenised inoculum is then aseptically transferred to the Erhlenmeyer flask which is incubated at 25°C under static conditions for a further 15 days.

Mevinolin is assayed in both the broth and methanolic extracts of the separated fungal biomass using established procedures employing high performance liquid chromatography (HPLC). Using this method, average levels of mevinolin in the biomass extracts have been recorded as 414 mg/l, while the average level measured in the cell-free fermentation broth was 224 mg/l.

The recorded levels of mevinolin produced using this procedure are comparable with standard inoculation procedures using separate liquid fermentations (usually

using a different medium composition) to generate inoculum for the production medium. The mevinolin can then be isolated and encapsulated for human consumption in accordance with established procedures.

The foregoing description demonstrates that the illustrated embodiment is capable of being used to maintain an organism, of a filamentous nature, by repeated subculturing without suffering from the effects of genetic segregation. Moreover, contamination can be limited by maintaining sterility of the body and the caps, and by exercising caution when connecting and disconnecting bodies from each other.

A wide range of growth media can be used with the apparatus of the illustrated embodiment. However, it will be appreciated that further growth media, including synthetic growth media, could also be used in conjunction with the apparatus. As synthetic growth media improve, their use may be desirable in terms of cost, reliability and sterility.

By using vessels of different lengths, sub-samples of the entire population can be effected easily. By using a particularly short vessel at a particular point in a chain of vessels, a small section of growth medium can be removed from the chain for further analysis of the organism residing therein. By taking a sample at or near to the growing end of the organism, the most viable biomass can be removed and used in inoculation of further apparatus.

CLAIMS:

1. A process for growing an organism, comprising:

providing a first vessel (30) containing a first body of growth supporting material (50) and causing the organism to grow in said material in said first vessel towards a first location;

providing a second vessel (30) containing a second body of growth supporting material (50); and

permitting said organism to grow from the body of material (50) in the first vessel (30) into the body of material (50) in the second vessel (30) through said first location.

2. A process according to claim 1, comprising connecting said second vessel (30) to said first vessel (30) and permitting said organism to grow into said second body (50) of material while said vessels are connected together.

3. A process according to claim 2 wherein said connecting step is performed in a sterile manner.

4. A process according to claim 2 or claim 3, comprising disconnecting said first vessel (30) from said

second vessel (30) after said organism has begun to grow in said second body of material.

5. A process according to claim 4 wherein said disconnecting step is performed in a sterile manner.

6. A process according to any one of claims 1 to 5 wherein said step of causing said organism to grow comprises causing said organism to grow in said first vessel (30) and into said second vessel (30) in a predetermined growing direction.

7. A process according to claim 6 wherein said first and second vessels (30) are, in the predetermined direction, of different lengths.

8. A process according to claim 7 wherein said second vessel (30) is shorter than said first vessel (30), said process further comprising said step of removing said second vessel, after said step of permitting said organism to grow therein, for sub-sampling thereof.

9. A process according to any one of claims 1 to 8, comprising causing said organism to grow in said second body of material (50) towards a second location therein,

providing a third vessel (30) containing a third body of growth supporting material (50) and permitting said organism to grow from said second body of material (50) into said third body of material (50) through said second location.

10. A process of storing a microorganism comprising the steps of:

providing a growth medium (50);

growing a population of a microorganism on or in the growth medium (50); and

sampling said population for subculture;

characterised in that said step of sampling includes sampling across substantially the whole population of the microorganism.

11. A process of storing a microorganism including the steps of:

providing a growth medium (50); and

causing a microorganism to grow on or in the growth medium; characterised in that said step of causing said organism to grow includes training said microorganism substantially in a predetermined direction.

12. A process in accordance with claim 11 wherein said

step of causing said organism to grow includes causing said organism to grow towards a predetermined location, and said method further comprising sampling at said predetermined location to obtain a sample of said organism across substantially the entire population thereof.

13. A process in accordance with claim 12, wherein said sampling step includes the step of placing a sampling medium (50) adjacent said predetermined location for continuing growth of said microorganism thereon or therein.

14. A process of manufacturing a metabolite comprising the steps of:

storing a microorganism in accordance with the method of any of claims 1 to 13;

extracting a sample of said microorganism;

subjecting said sample to conditions suitable for metabolism; and

extracting metabolite from said sample.

15. A method of preparing a pharmaceutical preparation including the step of isolating a metabolite produced in accordance with claim 14.

16. A pharmaceutical preparation including a metabolite the product of a method in accordance with claim 14.

17. A storage device for use in the process of any of claims 1 to 15 including a housing (30), growth medium (50) within the housing (32), and first and second locations on the growth medium, such that a microorganism can be grown from the first location towards the second location where subculturing of substantially the entire population can be effected.

18. A storage device in accordance with claim 17, wherein the housing (32) is tubular.

19. A storage device in accordance with claim 18, wherein the housing (32) is cylindrical.

20. A storage device in accordance with any one of claims 17 to 19 wherein the housing (32) is of a sterilizable material.

21. A storage device in accordance with any one of claims 17 to 20 wherein said housing (32) has formations (34) at said first and second locations of the growth

medium each formation being suitable to engage with a cooperating formation of another of said storage device (30), for propagation of microorganism therebetween.

22. A storage device in accordance with any one of claims 17 to 21 wherein said housing (32) comprises means (40) for retaining said growth medium.

23. A storage device in accordance with claim 22 wherein said retaining means (40) has at least one aperture defined therein for passage of microorganism therethrough.

24. A storage device in accordance with claim 23 wherein said retaining means (40) comprises at least one retaining member (42') across said vessel.

25. A storage device in accordance with claim 24 wherein said retaining means (40) comprises a reticular member (42) across said vessel.

26. A storage device in accordance with any one of claims 17 to 25 wherein said growth medium (50) comprises a natural foodstuff.

27. A storage device in accordance with claim 26 wherein said foodstuff is a vegetal foodstuff.

28. A storage device in accordance with claim 27 wherein said growth medium (50) comprises a quantity of a cereal.

29. A storage device in accordance with claim 27 or claim 28 wherein said growth medium (50) comprises a quantity of seed.

30. A storage device in accordance with any one of claims 27, 28 or 29 wherein said growth medium (50) comprises a quantity of a pulse.

31. A storage device in accordance with any one of claims 27 to 30 wherein said growth medium (50) comprises an agricultural crop byproduct.

32. A storage device in accordance with claim 31 wherein said agricultural crop byproduct comprises at least one of ground corn cobs, peanut shells, tea leaves and straw.

33. A storage device in accordance with any one of claims 26 to 32 wherein said growth medium (50) comprises at least one of calcium sulphate, soy oil, yeast extract

and peptone.

34. A storage device in accordance with any one of claims 17 to 33 wherein said growth medium (50) is sterile.

35. A storage container for use in the storage device (30) of any one of claims 17 to 35, defining a cavity within which growth medium can be contained, said container (32) comprising first and second access means between which growth medium can extend in use, for growth of an organism between said first and second access means in use.

36. A container in accordance with claim 35 including first and second closure means (30), removably closing said access means in use.

37. A storage device for use in the process of any one of claims 1 to 15 comprising:

growth medium (50) for viably supporting a microorganism;

characterised in that

said storage device includes a facility for presenting said population substantially in its entirety

for subculture.

38. A storage device in accordance with claim 37 and further comprising a receptacle (32) supporting the growth medium.

39. A storage device in accordance with claim 38, wherein the growth medium defines a substantially elongate growing path.

40. A storage device in accordance with claim 38 or claim 39, wherein the receptacle (32) includes attachment means (34) for attachment of said device to further culturing apparatus.

41. A storage device in accordance with claim 40, wherein the attachment means (34) is operable to engage the growth medium with growth medium of a further storage device in accordance with any one of claims 37 to 40.

FIG.1

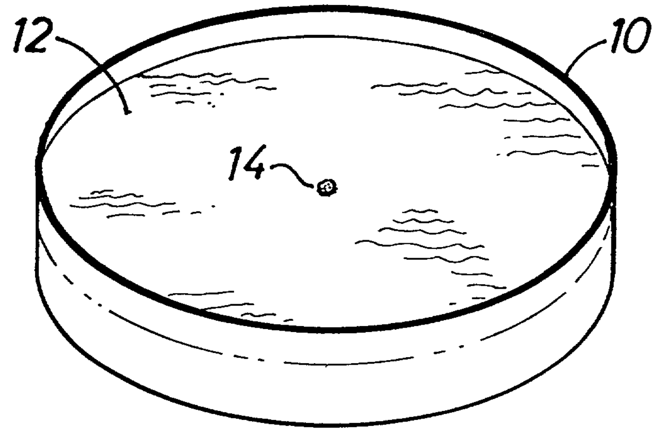


FIG.2

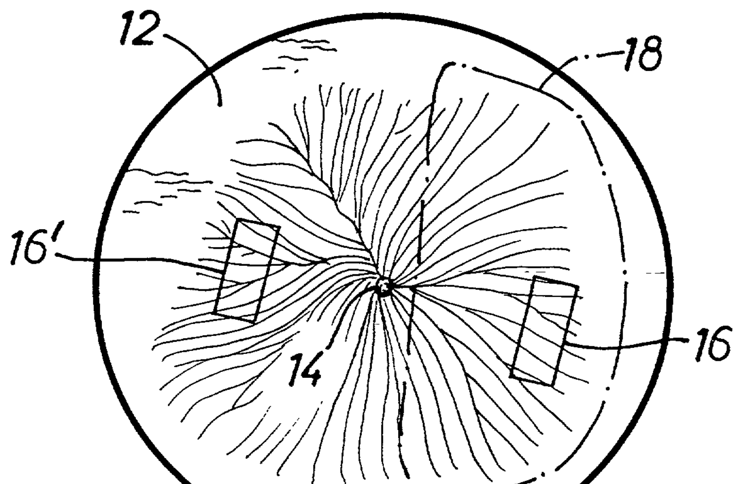


FIG. 3

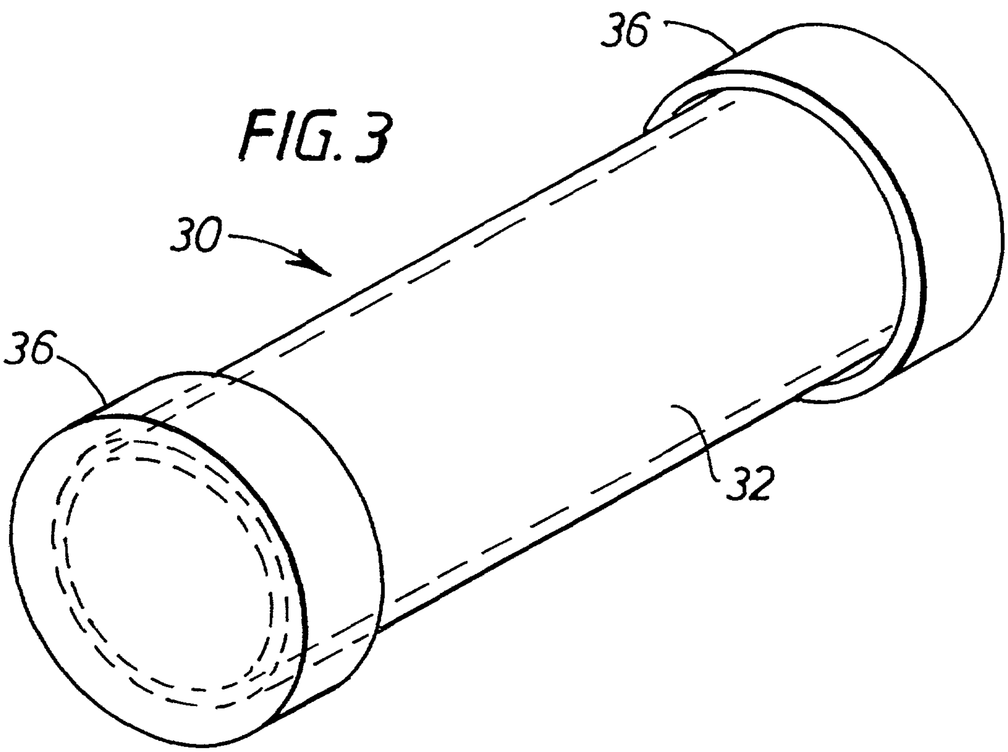


FIG. 4

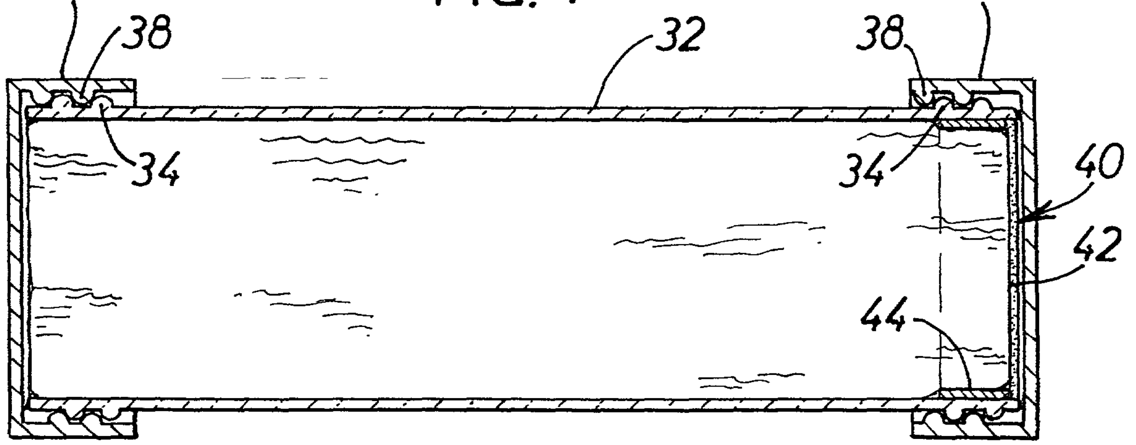


FIG.5

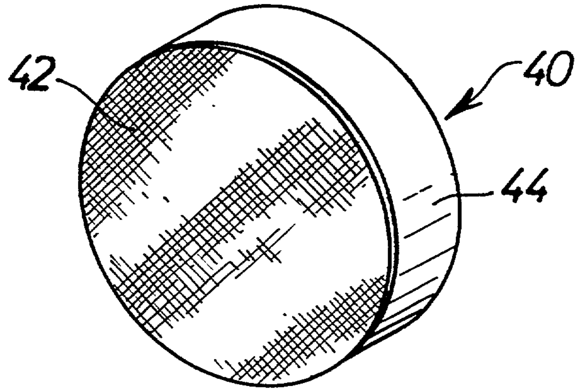
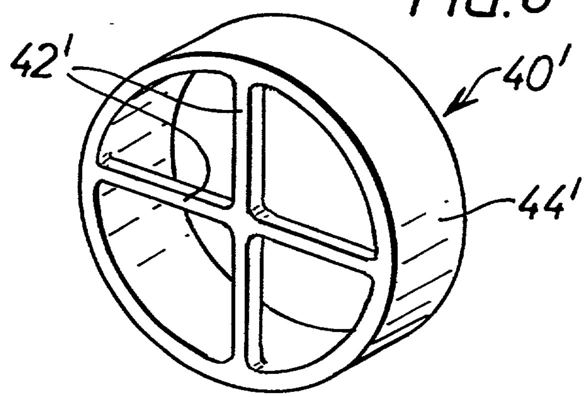


FIG.6



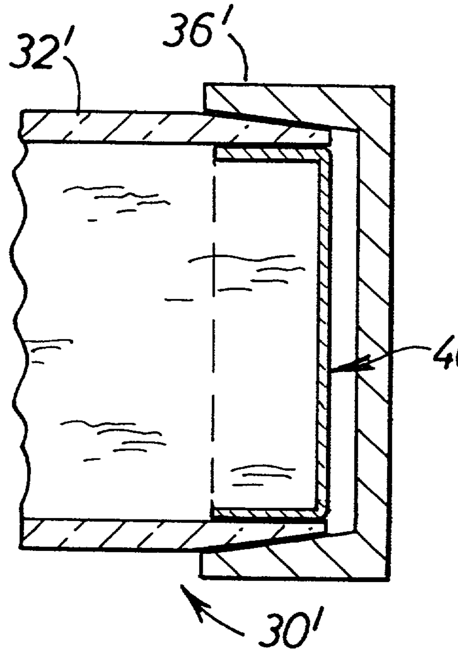
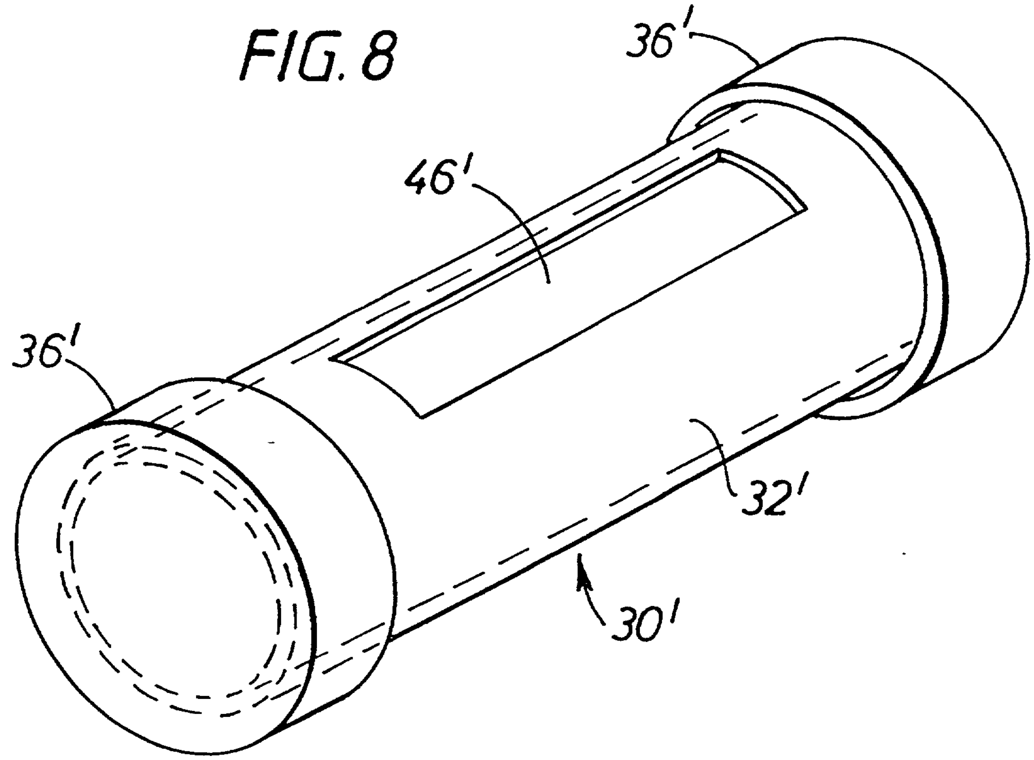


FIG.7

FIG. 8



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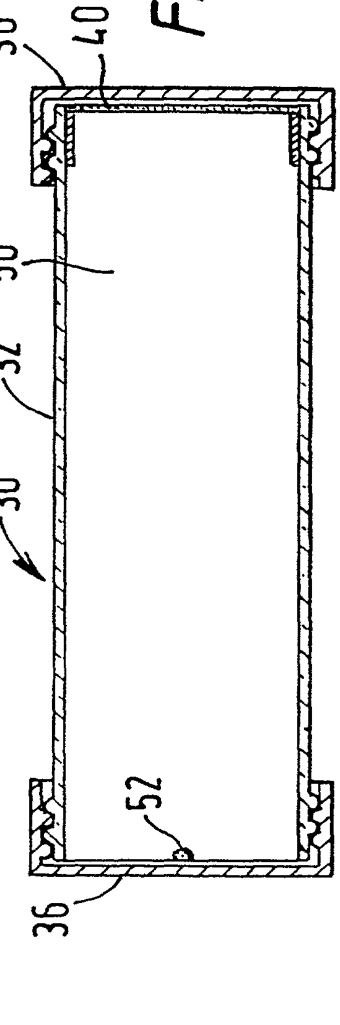

FIG. 9

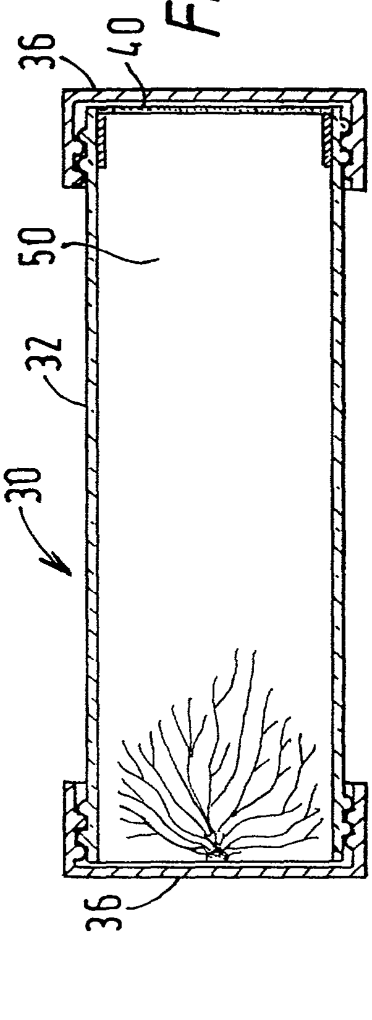

FIG.10

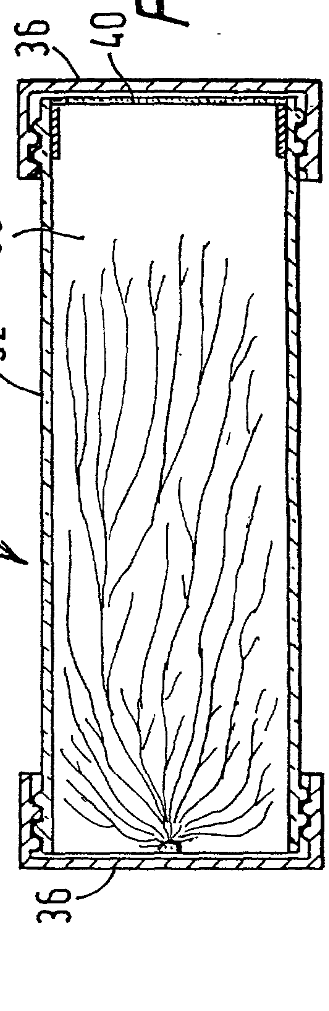

FIG.11

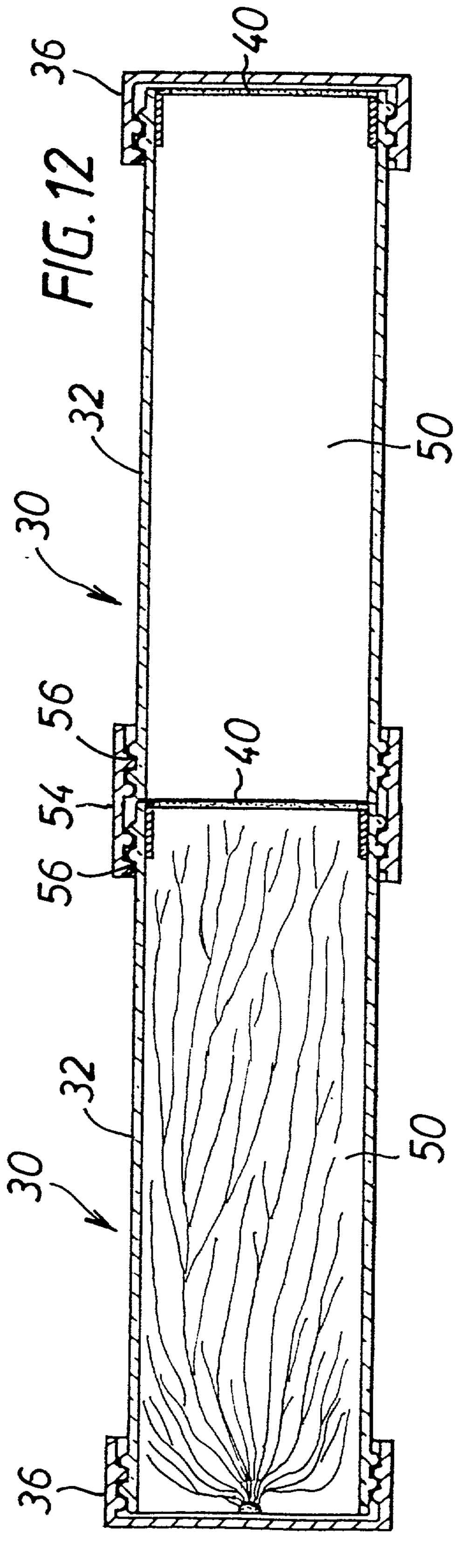

FIG. 12

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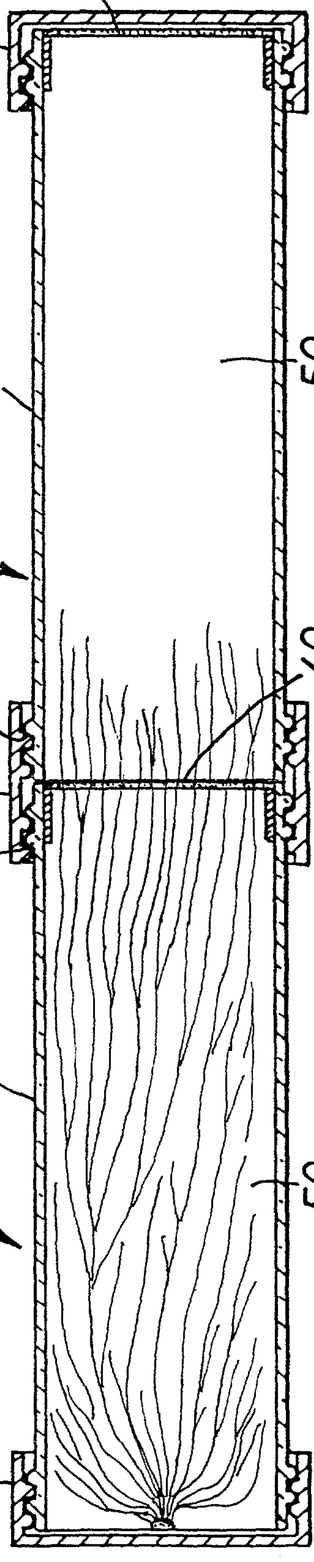

FIG.13

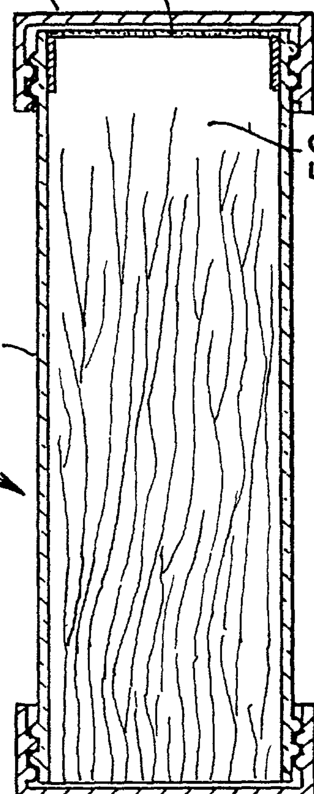

FIG.14

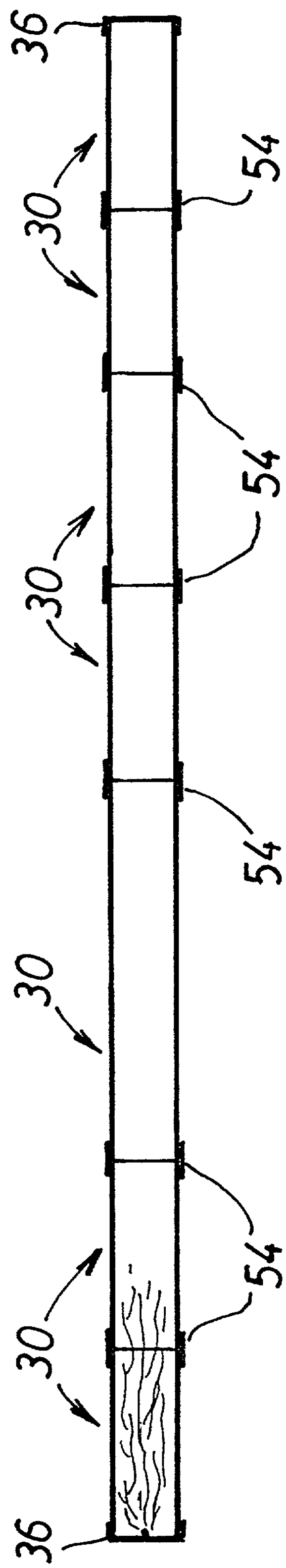

FIG.15

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: APPARATUS FOR PRESERVING MICROORGANISMS the specification of which ☐ is attached and/or **X** was filed on August 7, 2001 as United States Application Serial No. ____________________ and/or was filed on February 8, 2000 as PCT International Application No. PCT/GB00/00378 and was amended on ____________________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States, listed below and have also identified below, any foreign application(s) for patent or inventor's certificate, or any PCT International application(s) having a filing date before that of the application(s) of which priority is claimed:

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C. 119
United Kingdom	9902757.5	February 8, 1999	X YES ☐ NO
			☐ YES ☐ NO

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Number	Date of Filing

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application(s) and the national or PCT International filing date of this application:

Application Number	Date of Filing	Status (Patented, Pending, Abandoned)

I hereby appoint the following attorney and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., CUSTOMER NUMBER 22,852**, Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W. Edmondson, Reg. No. 33,871; Michael R. McGurk, Reg. No. 32,045; Joann M. Neth, Reg. No. 36,363; Gerson S. Panitch, Reg. No. 33,751; Cheri M. Taylor, Reg. No. 33,216; Charles E. Van Horn, Reg. No. 40,266; Linda A. Wadler, Reg. No. 33,218; Jeffrey A. Berkowitz, Reg. No. 36,743; Michael R. Kelly, Reg. No. 33,921; James B. Monroe, Reg. No. 33,971; Doris Johnson Hines, Reg. No. 34,629; Allen R. Jensen, Reg. No. 28,224; Lori Ann Johnson, Reg. No. 34,498; and David A. Manspeizer, Reg. No. 37,540 and ____________________. Please address all correspondence to **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.**, 1300 I Street, N.W., Washington, D.C. 20005, Telephone No. (202) 408-4000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Residence		Citizenship
Post Office Address		

Full Name of Fifth Inventor	Inventor's Signature	Date
Residence		Citizenship
Post Office Address		

Full Name of Sixth Inventor	Inventor's Signature	Date
Residence		Citizenship
Post Office Address		

Full Name of Seventh Inventor	Inventor's Signature	Date
Residence		Citizenship
Post Office Address		

Full Name of Eighth Inventor	Inventor's Signature	Date
Residence		Citizenship
Post Office Address		